

# CONTENTS

	<u>Page No.</u>
<b>CHAPTER 1: INTRODUCTION</b> .....	1
<b>CHAPTER 2: REVIEW OF LITERATURE</b> .....	4
2.1. Chlorophyll as a natural source of colour.....	4
2.1.1. Structure of chlorophyll pigments.....	4
2.1.2. Extraction and separation of chlorophyll pigments.....	4
2.1.3. Factors affecting the stability of chlorophyll .....	8
2.1.3.1. Effect of temperature: .....	8
2.1.3.2. Effect of pH .....	9
2.1.3.3. Effect of enzyme .....	10
2.1.3.4. Effect of metals .....	11
2.1.4. Preservation of chlorophylls .....	11
2.1.5. Determination of chlorophyll pigments .....	12
2.1.6. Application of chlorophyll pigments in food processing ...	13
2.2. Anthocyanin pigments as a natural source of colour .....	14
2.2.1. Structure of anthocyanin pigments .....	17
2.2.2. Extraction and separation of anthocyanin pigments .....	19
2.2.3. Factors affecting the stability of anthocyanin pigments ....	21
2.2.3.1. Effect of temperature .....	23
2.2.3.2. Effect of pH on pigment stability .....	25
2.2.3.3. Effect of sulphur dioxide on the stability of anthocyanin pigments .....	27
2.2.3.4. Effect of enzymes on the stability of anthocyanins .....	30
2.2.4. Application of anthocyanins pigments in food processing..	30
2.3. Carotenoid pigments as a natural source of colour .....	32

2.3.1. Structure of carotenoid pigments .....	32
2.3.2. Extraction and separation of carotenoid pigments .....	34
2.3.3. Factors affecting the stability of carotenoid pigments .....	35
2.3.3.1. Effect of temperature .....	35
2.3.3.2. Effect of oxygen on the carotenoid pigments .....	36
2.3.3.3. Effect of pH on the carotenoid pigments .....	36
2.3.4. Determination of carotenoid pigments .....	36
2.3.5. Application of carotenoid pigments in food processing ...	37
2.4. Microbiological characteristics of ice cream .....	38
2.5. Microbiological characteristics of fundan .....	39
<b>CHAPTER 3: MATERIALS AND METHODS.....</b>	<b>40</b>
3.1. Materials.....	40
3.2. Methods.....	40
3.2.1. Technological methods .....	40
3.2.1.1. Fundan preparation .....	40
3.2.1.2. Ice cream preparation .....	41
3.2.2. Chemical analysis .....	41
3.2.2.1. Extraction of chlorophyll pigments .....	41
3.2.2.2. Determination of chlorophyll pigments .....	41
3.2.2.3. Extraction of anthocyanin pigments .....	42
3.2.2.4. Determination of anthocyanin pigments .....	42
3.2.2.5. Extraction of carotenoid pigments .....	43
3.2.2.6. Determination of carotenoid pigments .....	43
3.2.3. Colour measurement .....	43
3.2.4. pH measurement .....	44
3.2.5. Effect of blanching .....	44
3.2.6. Effect of sodium carbonate .....	44

3.2.7. Effect of synthetic antioxidant .....	44
3.2.8. Effect of natural antioxidant .....	44
3.2.9. Effect of sulphur dioxide .....	45
3.2.10. Effect of ethylene diamine tetra acetic acid (EDTA) .....	45
3.2.11. Microbiological analysis .....	45
3.2.11.1. Total microbial count .....	45
3.2.11.2. Yeasts and mold count .....	45
3.2.12. Sensory evaluation .....	46
<b>CHAPTER 4: RESULTS AND DISCUSSION .....</b>	<b>47</b>
4.1. Factors affecting the stability and content of chlorophyll pigments .....	47
4.1.1. Effect of storage periods at different temperatures on total chlorophyll stability and content .....	47
4.1.2. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments ...	47
4.1.3. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments in alkaline medium .....	50
4.1.4. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments in alkaline medium with adding synthetic antioxidant (BHT).....	52
4.1.5. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments in alkaline medium with adding natural antioxidant (rosmarry) .....	52
4.1.6. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments in	

alkaline medium with adding synthetic antioxidant and storing under nitrogen .....	55
4.1.7. Effect of storage periods at different temperatures on the stability and content of blanched chlorophyll pigments in alkaline medium with adding natural antioxidant and storing under nitrogen .....	57
4.1.8. Effect of different pH values on chlorophyll stability .....	57
4.1.9. The stability and content of chlorophyll colour in some food products as fundan and ice cream .....	60
4.1.10. Sensory evaluation of fundan and ice cream coloured with green pigment extracted from chard leaves .....	62
4.2. Factors affecting the stability and content of anthocyanin pigments .....	64
4.2.1. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from strawberries (control) .....	64
4.2.2. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from strawberries treated with ethylene diamine tetra acetic acid (EDTA) .....	67
4.2.3. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched strawberries .....	67
4.2.4. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched strawberries treated with sulphur dioxide .....	70



4.2.5. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched strawberries treated with natural antioxidant .....	70
4.2.6. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched strawberries with adding sulphur dioxide and natural antioxidant .....	72
4.2.7. Effect of extraction at high pH, using sodium carbonate and citrate buffer solution, on the stability of blue anthocyanin pigments extracted from blanched strawberries at the alkaline state .....	75
4.2.8. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from rosella flowers (control) .....	75
4.2.9. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched rosella flowers .....	77
4.2.10. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched rosella flowers treated with sulphur dioxide .....	77
4.2.11. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments extracted from blanched rosella flowers treated with natural antioxidant. ....	80
4.2.12. Effect of storage periods at different temperatures on the stability and content of red anthocyanin pigments	

extracted from blanched rosella flowers with adding sulphur dioxide and natural antioxidant .....	80
4.2.13. Effect of alkaline state by adding sodium carbonate and citrate buffer solution on the stability of the formed blue anthocyanin pigments extracted from blanched rosella flowers .....	83
4.2.14. Effect of storage periods at different temperatures on the stability and content of violet- anthocyanin pigments extracted from beet root (control) .....	83
4.2.15. Effect of storage periods at different temperatures on the stability and content of violet- anthocyanin pigments extracted from blanched beet root .....	85
4.2.16. Effect of storage periods at different temperatures on the stability and content of violet- anthocyanin pigments extracted from blanched beet root treated with sulphur dioxide .....	85
4.2.17. Effect of storage periods at different temperatures on the stability and content of violet anthocyanin pigments extracted from blanched beet root treated with natural antioxidant .....	88
4.2.18. Effect of storage periods at different temperatures on the stability and content of violet anthocyanin pigments extracted from blanched beet root with adding sulphur dioxide and natural antioxidant .....	88
4.2.19. Effect of extraction at high pH, using sodium carbonate and citrate buffer solution, on the stability of blue anthocyanin pigments extracted from blanched beet root at the alkaline state .....	91

4.2.20. Effect of different pH values on the stability and content of anthocyanin pigments .....	91
4.2.21. The stability of anthocyanin pigments from some fruits as used in fundan and ice cream processing .....	93
4.2.22. Sensory evaluation of fundan and ice cream coloured with anthocyanin pigments extracted from: strawberries, rosella flowers, and beet root .....	95
4.3. Factors affecting the stability and content of carotenoid pigments.....	102
4.3.1. Effect of storage periods at different temperatures on the stability and content of orange carotenoid pigments extracted from orange peel (control) .....	102
4.3.2. Effect of storage periods at different temperatures on the stability and content of orange carotenoid pigments extracted from blanched orange peel .....	102
4.3.3. Effect of storage periods at different temperatures on the stability and content of orange carotenoid pigments extracted from blanched orange peel with adding sulphur dioxide .....	105
4.3.4. Effect of storage periods at different temperatures on the stability and content of orange carotenoid pigments extracted from blanched orange peel with adding natural antioxidant .....	105
4.3.5. Effect of storage periods at different temperatures on the stability and content of orange carotenoid pigments extracted from blanched orange peel with adding sulphur dioxide and natural antioxidant .....	108

4.3.6. Effect of storage periods at different temperatures on the stability and content of yellow carotenoid pigments extracted from carrot .....	110
4.3.7. Effect of storage periods at different temperatures on the stability and content of yellow carotenoid pigments extracted from blanched carrot .....	110
4.3.8. Effect of storage periods at different temperatures on the stability and content of yellow carotenoid pigments extracted from blanched carrot treated with sulphur dioxide .....	113
4.3.9. Effect of storage periods at different temperatures on the stability and content of yellow carotenoid pigments extracted from blanched carrot with adding natural antioxidant .....	113
4.3.10. Effect of storage periods at different temperatures on the stability and content of yellow carotenoid pigments extracted from blanched carrot with adding sulphur dioxide and natural antioxidant .....	116
4.3.11. Effect of different pH values on the stability and content of carotenoid pigments .....	116
4.3.12. The stability of carotenoid pigments in fundan and ice cream .....	119
4.3.13. Sensory evaluation of fundan and ice cream coloured with carotenoid pigments extracted from blanched carrot with adding sulphur dioxide and natural antioxidant .....	119
4.3.14. Sensory evaluation of fundan and ice cream coloured with carotenoid pigments extracted from blanched carrot with adding sulphur dioxide and natural antioxidant.....	123



4.3.15. Microbiological characteristics of ice cream coloured with different natural pigments .....	129
4.3.16. Microbiological characteristics of fundan coloured with different natural pigments .....	129
<b>CHAPTER 5: SUMMARY AND CONCLUSION .....</b>	<b>136</b>
<b>CHAPTER 6: REFERENCES .....</b>	<b>142</b>
<b>CHAPTER 7: ARABIC SUMMARY .....</b>	

## CHAPTER 5

### SUMMARY AND CONCLUSION

The importance of adding colours to reinforce colours already present in food but less intense than the consumer would expect.

- To ensure uniformity of colour in food during industry
- To restore the original appearance of food whose colour has been affected by processing.
- To give colour to certain foods as sugar confectionery, ice lollies and soft drinks, which would otherwise be virtually colourless.

During this century, the use of synthetic colours has steadily increased due principally to their ready availability and lower relative price. In the last 20 years, there was an increasing trend in food to use the natural pigments due to its safety of health and nutrition. Therefore, the aim of the present investigation was to improve the functional and sensorial of natural pigments through some technical treatments which will increase its incorporation into Egyptian foods during industry of foods. Extraction of natural pigments from different parts of plant with treating it by heating, adding sodium carbonate, sulphur dioxide, synthetic antioxidant and natural antioxidant. The relationship between pH and the changing of extracted pigments colour and quantity. It was used the extracted pigments to colour some processing foods (Fundan and ice cream) and study the stability of this pigments in foods which were subjected to sensory evaluation. Also, microbiological characteristics of fundan and ice cream were discussed during storage periods.

The obtained results of the present study can be summarized in the following points:

1. The results indicated that chlorophyll pigment extracted from blanched chard leaves with adding sodium carbonate and natural antioxidant under nitrogen was stable for a long time without change during storage periods at different temperatures. The green colour of pigment was stable for 9 days at  $-18^{\circ}\text{C}$ , 6 days at  $4^{\circ}\text{C}$  and 4 days at  $25^{\circ}\text{C}$ . There was a proportional relation between storage periods at different temperatures and degradation rate of pigment colour.
2. The obtained results were revealed that red anthocyanin colour extracted from blanched strawberries with adding sulphur dioxide and natural antioxidant was stable for 32 days at  $-18^{\circ}\text{C}$ , 24 days at  $4^{\circ}\text{C}$  and 16 days at  $25^{\circ}\text{C}$ . The destruction of red colour showed a gradual proportional stability and quantity with temperature and holding time of anthocyanin solution.
3. Red anthocyanin pigments extracted from blanched rosella flowers with adding sulphur dioxide and natural antioxidant were stable for 28 days at  $-18^{\circ}\text{C}$ , 20 days at  $4^{\circ}\text{C}$  and 12 days at  $25^{\circ}\text{C}$ .
4. Violet anthocyanin pigments extracted from blanched beet root with adding sulphur dioxide and natural antioxidant was stable for up to 28 days at  $-18^{\circ}\text{C}$ , 20 days at  $4^{\circ}\text{C}$  and 12 days at  $25^{\circ}\text{C}$ .
5. Blue anthocyanin pigments was extracted at high pH value using sodium carbonate and citrate buffer solution from blanched strawberries, rosella flowers and beet root at the alkaline state, were unstable and the colour was lost quickly. The stability of blue colour extracted from rosella flowers was more stable than blue colour extracted from strawberries and beet root. The colour was stable for 36, 15 and 10 hours on storing at  $-18^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ , respectively.

6. Orange carotenoid pigments extracted from blanched orange peel with adding sulphur dioxide and natural antioxidant were stable for 36 days at  $-18^{\circ}\text{C}$ , 24 days at  $4^{\circ}\text{C}$  and 16 days at  $25^{\circ}\text{C}$ .
7. Yellow carotenoid pigments extracted from blanched carrot with adding sulphur dioxide and natural antioxidant were stable for 32, 20 and 12 days on storing at  $-18^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ , respectively. The previous results indicated that the loss of chlorophyll pigments was higher and quicker than the loss of anthocyanin pigments or carotenoid pigments, while the blue anthocyanin pigments were very unstable.
8. The data obtained for studying the effect of different pH values on the stability of extracted pigments were as followed:
  - a. Chlorophyll quantity values was 45.66 mg/L at pH 9, while this values decreased on decreasing the pH. The green colour was still stable at pH 7, 8 and 9 then it changed to olive-green colour with decreasing in pH.
  - b. Total anthocyanin values decreased with increasing the pH values. Anthocyanin content extracted from strawberries decreased from 151.0 mg/100 g to 29.0 mg/100 g, on increasing the pH from 2 to 10, respectively. The colour changed from light-red to red, blue and brown, while, corresponding values in rosella flowers decreased from 110.0 mg/100 g to 23.0 mg/100 g, and the colour changed from red to blue, to brown colour at pH 10. The values of anthocyanin pigments in beet root was 100.0 mg/100 g and decreased to 75.0 mg/100 g with increasing pH from 2 to pH 10, and the colour changed from violet to blue to brown.
  - c. Total carotenoids quantity values was higher at pH 7 and 8, and decreased on decreasing pH values of pigments extracted from



orange peel. At pH 7 the content of pigments was 100.0 mg/100 g and at pH 8 it was 115.0 mg/100 g. The orange colour was lost on decreasing pH values. The quantity values of carotenoid pigments extracted from carrot decreased on decreasing pH values and the colour changed from yellow to light yellow.

9. The results of colour characteristics for some food processing (fundan and ice cream) containing the studied pigments:
  - a. Tests were applied on using chlorophyll pigments extracted from blanched chard leaves in alkaline medium with adding natural antioxidant on colouring fundan and ice cream. The green colour in fundan was stable for 12 days at room temperature and brightness decreased from 56.33% to 26.92%. In ice cream, the colour was kept for 3 weeks at freezing temperature, the brightness decreased from 57.59% to 30.33%.
  - b. Data revealed that adding anthocyanins pigments extracted from blanched strawberries with adding sulphur dioxide and natural antioxidant to fundan and ice cream improved the stability of colour in fundan for 3 weeks and the brightness of red colour was 37.25%. While, in case of ice cream red colour was stable for 6 weeks and the brightness decreased from 37.25% to 20.12% during storage periods.
  - c. Adding red anthocyanin pigments extracted from blanched rosella flowers with adding sulphur dioxide and natural antioxidant to colour fundan and ice cream increased the stability of colour for 3 weeks in fundan, while the brightness decreased from 32.73% to 17.50%. The red colour was stable for 6 weeks in ice cream, while brightness decreased from 32.73% to 18.85% during storage.
  - d. Adding violet anthocyanin pigments extracted from blanched beet

root with adding sulphur dioxide and natural antioxidant to colour fundan and ice cream increased the stability of colour for 2 weeks in fundan at room temperature, while the brightness decreased from 32.73% to 16.0% and in ice cream the colour was stable for 4 weeks at freezing temperature, while the brightness decreased from 32.73% to 17.30%.

- e. Adding orange colour of carotenoid pigments extracted from blanched orange peel with adding sulphur dioxide and natural antioxidant to colour fundan and ice cream. The orange colour of fundan was stable for 3 weeks, while brightness decreased from 56.89% to 22.31% and in ice cream the orange colour was stable for 8 weeks while, the brightness decreased from 56.89% to 24.30%.
  - f. Adding yellow colour of carotenoid pigments extracted from blanched carrot with adding sulphur dioxide and natural antioxidant to colour fundan and ice cream, the yellow colour in fundan was stable for 2 weeks, while the brightness decreased from 55.62% to 18.60. The yellow colour was stable for 6 weeks in ice cream and the brightness decreased form 56.89% to 21.54%.
10. Data of sensory evaluation showed that adding extracted pigments to fundan and ice cream had improved most sensory attributes of ice cream as compared to fundan coloured with the same pigments.
  11. The results of microbiological characteristics of ice cream and fundan coloured with different natural pigments indicated that total bacterial and yeast and mold counts of ice cream were gradually decreased during storage at  $-18^{\circ}\text{C}$ , while small reduction in total bacterial counts and increases in yeast and mold counts were noticed.

According to the previously mentioned results, it can be concluded that:

- Treating chared leaves, strawberries, rosella flowers beet root orange peel and carrot by heating and adding sodium carbonate, sulphur dioxide, natural and synthetic antioxidants at different temperatures of storage is effective reducing degradation rates in extracted pigments and improving its colour stability and quantity for a long time of storage.
- Extracted pigments can be incorporated into some food processing in order to make a profit of its high quality colour, stability and safety for health.

Finally, from an economical and nutritional point of view, it is no doubt that using natural pigments is an inevitable for both producer and consumer as a good income and health benefits can be accomplished upon using natural pigments in the field of food technology.